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Phosphoinositide Binding Domains: Embracing 3-Phosphate

Minireview

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Perhaps the most dominant theme that has emerged in the past decade of signal transduction research is that proteins can become acutely targeted to new cellular locations (usually membranes) by phosphorylation reactions that create binding sites for modular protein domains. The classic example is the Src homology 2 (SH2) domain, which escorts signaling proteins to tyrosine-phosphorylated growth factor receptors or adaptor proteins.

In an analogous fashion, phosphorylation of the lipid phosphatidylinositol (PtdIns) can create sites for recruitment of proteins to cell membranes. The phosphorylation or hydrolysis of inositol-containing lipids in cell membranes is now known to orchestrate numerous complex cellular events (reviewed by Corvera and Czech, 1998; Rameh and Cantley, 1999). A variety of domains that recognize specific phosphoinositides (phosphorylated forms of PtdIns) have been described and include pleckstrin homology (PH) domains, FYVE domains, and subsets of gelsolin homology domains, SH2 domains, and PTB domains. Some of these domains exist as large families, and specificity in recruitment is achieved by the ability of individual members of the family to recognize the phosphorylated moiety within distinct structural contexts.

The unique structure of the myo-inositol headgroup of inositol-containing lipids, with six similar but nonequivalent hydroxy groups, provides a template for formation of multiple phosphorylated species. It is the ability of PtdIns to form so many distinct phosphorylation and hydrolysis products that has allowed it to evolve into a central regulator in eukaryotic cells. Thus, phosphorylation of PtdIns at distinct positions on the inositol ring by different kinases results in the production of unique lipids that modulate discrete cellular responses at cell membranes.

For nearly 40 years, it was assumed that only two phosphorylated forms of PtdIns, PtdIns-4-P and PtdIns-4,5-P_2, exist in vivo. The discovery of phosphoinositide 3-kinase (PI3K) as an oncoprotein-associated enzyme approximately a decade ago revealed the existence of three additional phosphoinositides, PtdIns-3-P, PtdIns-3,4-P_2, and PtdIns-3,4,5-P_3. In mammalian cells, PtdIns-3-P is typically 5% as abundant as PtdIns-4-P or PtdIns-4,5-P_2. PtdIns-3,4-P_2 and PtdIns-3,4,5-P_3 are nominally absent in quiescent cells and are less than 3% as abundant as PtdIns-4,5-P_2 at peak stimulation. More recently, two additional phosphoinositides were found in eukaryotic cells, PtdIns-5-P and PtdIns-3,5-P_2 (Dove et al., 1997; Rameh et al., 1997; Whiteford et al., 1997).

The recent identification of proteins that specifically bind to lipid products of PI3K allows us to explain the mechanisms by which PI3K activation leads to cell growth, survival, transformation, actin rearrangement, vesicle trafficking, and transcriptional regulation (Corvera and Czech, 1998; Rameh and Cantley, 1999 and references therein). What has remained elusive is the structural basis for the selection of products of PI3K over the far more abundant phosphoinositides, PtdIns-4-P and PtdIns-4,5-P_2. Three recent publications (Baraldi et al., 1999; Kutateladze et al., 1999; Misra and Hurley, 1999) have revealed structures of phosphoinositide-binding proteins that begin to explain how cellular proteins can distinguish among different phosphoinositides. A crystal structure and a solution structure of two different FYVE domains indicate how these domains select for PtdIns-3-P, and a crystal structure of a PH domain reveals a mechanism for selective binding of PtdIns-3,4,5-P_3.

Structures of FYVE Domains

FYVE domains were so named based on the founding members of this family (*F*ab1, *Y*GLO23, *V*PS27, and *E*EA1) (Stenmark et al., 1996). These domains are small ($<$80 amino acids) and have two zinc coordination centers with structural similarities to sites in RING finger domains. However, members of this family have unique characteristics that set them apart from other RING finger domains, including a highly basic sequence motif (R/K-R/K-H-H-C-R) that is conserved from yeast to mammals. The yeast proteins that have FYVE domains are implicated in vesicle trafficking from the Golgi to the vacuole, downstream of the yeast PI3K homolog VPS34p (reviewed by Wurmser et al., 1999). The mammalian EEA1 protein is an early endosome protein involved in Rab5-dependent targeting and fusion of early endosomes, a process in which mammalian PI3Ks have been implicated (Li et al., 1995). Since VPS34p synthesizes PtdIns-3-P but cannot produce PtdIns-3,4-P_2 or PtdIns-3,4,5-P_3, PtdIns-3-P was implicated as the mediator of vesicle trafficking events in yeast. Last year three laboratories showed that FYVE domains from distinct proteins, including mammalian EEA1 and yeast VPS27p, bound specifically to vesicles containing PtdIns-3-P but not to vesicles containing other PIs (Burd and Emr, 1998; Gaullier et al., 1998; Patki et al., 1998; reviewed in Wurmser et al., 1999). Mutations within the basic motif reduced the ability to bind to PtdIns-3-P, suggesting that this region is required for lipid binding.

The crystal structure of the VPS27p FYVE domain solved by Misra and Hurley (1999) reveals that the basic motif conserved in FYVE domains and implicated in PtdIns-3-P binding forms a highly charged basic pocket on the surface of the domain. The overall structure of this domain is similar to that of the zinc-binding domains of rabphilin 3A and protein kinase C (PKC) family members. Two small β sheets (each containing only two strands) are held in place by the zinc coordination and

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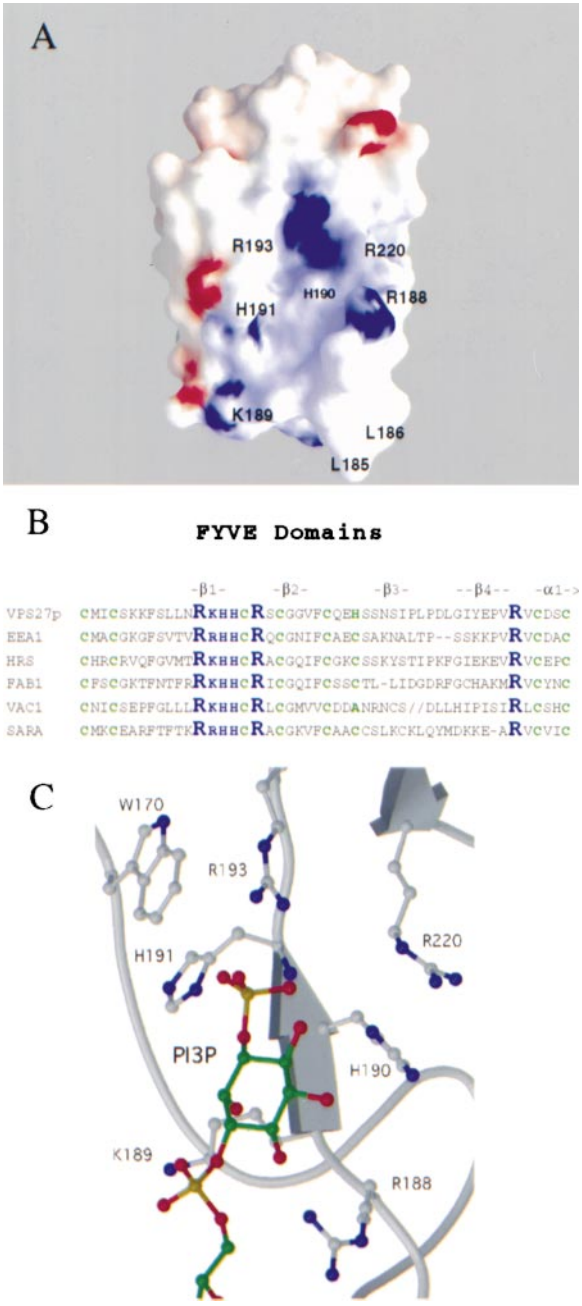


Figure 1. PtdIns-3-P-Binding Pocket of FYVE Domains

(A) Crystal structure of the VPS27p FYVE domain. The protein is shown as a GRASP structure, and the residues of the basic motif characteristic of these domains are indicated. The blue color indicates basic regions. Adapted by Dr. M. Yaffe based on structure from Misra and Hurley (1999).

(B) The sequence alignment of VPS27p FYVE domain with other FYVE domains. The most highly conserved core region of FYVE finger domains is presented. Basic residues implicated in PtdIns-3-P binding are indicated in blue. Arg-188, Arg-193, and Arg-220 of VPS27p (analogous to Arg-1369, Arg-1374, and Arg-1399 of EEA1), proposed to coordinate the phosphates of PtdIns-3-P, are indicated in larger font. The residues that coordinate the two zinc atoms are in green. Regions of α helix and β sheet structures in the VPS27p crystal structure are indicated. VPS27p, HRS, EEA1, FAB1, and VAC1 FYVE domains have been shown to bind specifically to PtdIns-3-P.

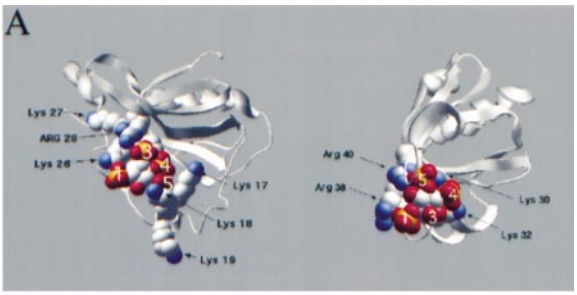
capped by a C-terminal α helix. The basic motif is mostly within the β1 strand that leads into a β turn containing one of the Cys pairs involved in zinc coordination (Figure 1B). Figure 1A presents the surface potential of the VPS27p FYVE domain from a view that faces the edge of the β1 strand. From this view, side chains of sequential residues appear on opposite sides of the β1 strand, and the backbone of β1 forms the central indentation of the pocket. In addition to the five basic residues from the motif, a sixth residue (Arg-220) from β4 completes the basic pocket.

Based on mutational data indicating residues involved in PtdIns-3-P binding (Burd and Emr, 1998) and by analogy to the PKCδC1 domain/phorbol ester–binding pocket, Misra and Hurley proposed a model for PtdIns-3-P binding specificity of the VPS27p FYVE domain (Figure 1C). In this model, the inositol ring sits in the central indentation of β1 and the 3-phosphate interacts with the aromatic ring of His-191 and the guanidino moiety of Arg-193, while the 1-phosphate forms a salt bridge with Arg-188. This model can explain the exclusion of more highly phosphorylated forms of PtdIns, since the pocket is too small to accommodate molecules with equatorial phosphates at multiple sites of the inositol ring. The exclusion of PtdIns-4-P from the pocket can be rationalized, since the distance between the 1- and 4-phosphates of PtdIns-4-P is greater than between the 1- and 3-phosphates of PtdIns-3-P and thus too large for optimal coordination by the two basic clusters. This model indicates that the fatty acids of PtdIns-3-P orient in the direction of the two hydrophobic residues Leu-185 and Leu-186 (Figure 1A). The model suggests that these residues interact with the hydrophobic region of the lipid bilayer to provide additional affinity for the membrane lipid. All the residues proposed to interact with PtdIns-3-P are conserved in other FYVE domains that have been shown to be specific for this lipid (Figure 1B).

The model proposed by Misra and Hurley is supported by NMR chemical shift changes observed upon binding of dibutanoyl-PtdIns-3-P to the FYVE domain of EEA1 (Kutateladze et al., 1999). For example, Arg-1369, His-1372, Arg-1374, and Arg-1399 of EEA1 (analogous to Arg-188, His-191, Arg-193, and Arg-220 of VPS27p; Figures 1A and 1B) all undergo large chemical shift changes upon binding to PtdIns-3-P. The proposal that Leu-185 and Leu-186 of VPS27p insert into the hydrophobic region of the membrane is strongly supported by NMR data indicating that $^{1}H/^{15}N$ resonances of the analogous loop region of EEA1 (Phe–Ser–Val-1366–Thr-1367) disappear when EEA1 binds to micelles containing PtdIns-3-P but are not similarly affected by binding to soluble dibutanoyl-PtdIns-3-P. In addition, mutation of Val-1366–Thr-1367 of the EEA1 FYVE domain (analogous to Leu-185–Leu-186 of VPS27p) eliminated binding to the membrane in vitro and in vivo.

Interestingly, PtdIns-5-P (the closest structural homolog of PtdIns-3-P) was shown to induce similar chemical shift changes on EEA1 to those induced by PtdIns-3-P,

(C) A model for how the PtdIns-3-P headgroup interacts with the basic pocket of the FYVE domain. The view is the same as in (A). Adapted from Misra and Hurley (1999).



B

		--β1--	--β2---
GROUP I		3 5	3
		4 1 1	1
High Affinity	BTK	SIFL**K**RS..QQ**KK**TSPLN...F**KKR**LFLL	
PtdIns-3,4,5-P$_3$ or	GAP1	GFMI**K**RG..QG**KR**FGMKN...F**KKR**WFRL	
Ins-1,3,4,5-P$_4$	GAB1	GWLR**K**SP..PE**K**LKRYA....W**KR**WFVL	
Binding	GRP-1	GWLL**K**LG..GR**V**KT.....W**KR**WFIL	
Group II		5	5
		4 4 1	
PtdIns-4,5-P$_2$	PLC-δ	SQLL**K**VK..SSS.....W**RRER**FYKL	
or Ins-1,4,5-P$_3$			
(& PtdIns-3,4,5-P$_3$)			
Binding			
Group III			
PtdIns-3,4-P$_2$	PDK1	GFVL**K**RK..GL.....F**ARRR**QLLL	
or PtdIns-3,4,5-P$_3$	AKT/PKB	GWLH**K**RG..EYIKT.....W**RER**YFLL	
Binding			
Group IV			
Low Affinity	DYNAMIN	GWLTINN..IGIMK**G**G.....S**K**EYWFVL	

Figure 2. Phosphoinositide-Binding Pocket of PH Domains

(A) The crystal structure of the BTK PH domain bound to inositol-1,3,4,5-tetrakisphosphate (IP_4) is presented on the left, and the PH domain of PLCδ1 bound to inositol-1,4,5-trisphosphate is presented on the right. Basic residues from the β1-β2 loop region that interact with the phosphates numbered are indicated. Adapted by Dr. M. Yaffe based on structure from Baraldi et al. (1999).

(B) Alignment of residues from the β1-β2 region of the BTK PH domain with homologous regions of other Group I, Group II, Group III, and Group IV PH domains (adapted from Rameh et al., 1997). The numbers above residues of BTK and PLCδ PH domains indicate side chains that contact phosphates at the 1, 3, 4, or 5 positions of the inositol ring. Basic residues are indicated in blue. Lys-12 and Arg-28 of BTK, Lys-30 and Arg-40 of PLCδ, and analogous residues of the other PH domains are indicated in bold.

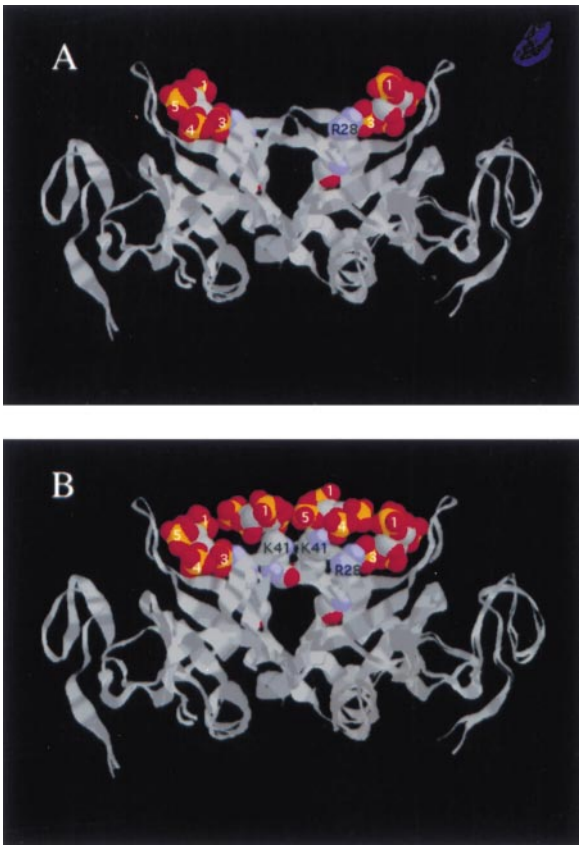


Figure 3. Dimers in the Crystal Structures of BTK PH Domains Bound to IP_4

(A) Wild-type BTK PH domain.

(B) E41K BTK PH domain, each bound to two molecules of IP_4. Note that each of the 1-phosphates points in the same direction, suggesting a mechanism for strengthening the avidity of the interaction with PIP_3-containing membranes. Adapted by Dr. M. Yaffe based on structure from Baraldi et al. (1999).

but at higher lipid concentrations. One might predict that PtdIns-5-P would bind to the same pocket as PtdIns-3-P but with the inositol ring flipped over 180° to allow the 5-phosphate to occupy the same location as the 3-phosphate (see discussion of PH domains below). However, since the 3-phosphate and 5-phosphate extend from the same side of the inositol ring, the inositol moiety would have to sit deeper in the pocket in the flipped orientation in order for the 1- and 5-phosphates to occupy the same locations as the 1- and 3-phosphates in the model of Figure 1C. Thus, high-affinity PtdIns-5-P binding may be precluded by steric hindrance.

Although PtdIns-3-P has been shown to play a role in vesicle trafficking by recruiting proteins to early endosomes in mammalian cells and to analogous structures in yeast, the discovery of a host of proteins with FYVE domains bearing the basic motif predictive of PtdIns-3-P binding suggests that this lipid may play a much larger role in cellular regulation than previously thought. For example, SARA is a FYVE domain–containing protein that has been shown to recruit SMAD domains to the TGFβ receptor (Tsukazaki et al., 1998). FGD-1, implicated in facial and genital development, has both a FYVE domain and a domain that mediates GTP/GDP exchange on CDC42 (Pasteris et al., 1994). Thus, it is possible that PtdIns-3-P-dependent recruitment of proteins to early endosomes is also important for initiating signaling events.

Ins-1,3,4,5-P$_4$/BTK PH Domain Crystal Structure

A variety of signaling proteins, including protein-Tyr kinases, Ser/Thr kinases, and GDP/GTP exchange factors have PH domains that are important for function (Corvera and Czech, 1998; Leevers et al., 1999; Rameh and Cantley, 1999). More than 100 PH domain–containing proteins are predicted based on sequence data banks. Most have not yet been characterized, but where investigated, phosphoinositides have been shown to bind to most of these domains (Rameh et al., 1997a). Those that have been characterized to date fall into four major groups in regard to their phosphate binding specificity (Figure 2B). The PH domains that bind with high specificity to PtdIns-3,4,5-P_3 or PtdIns-3,4-P_2 (Groups I and III, respectively) have been of particular interest in recent years because of their role in transmitting growth and survival signals downstream of PI3K.

Previous structures of PH domains bound to the headgroup of PtdIns-4,5-P_2, inositol-1,4,5-trisphosphate (IP_3), have provided information about residues involved in

headgroup recognition but have not revealed how discrimination among related phosphoinositides is accomplished. The most informative of these structures was the phospholipase C (PLC) δ PH domain/IP_3 complex (Figure 2A) (Ferguson et al., 1995). This structure revealed that the inositol moiety is sandwiched between the β1-β2 loop and the β3-β4 loop at one end of a twisted β barrel. Most of the contact with the phosphates occurs with basic residues from the β1 and β2 strands, most notably, Lys-30 from β1 and Arg-40 from β2 that interact with the 5- and 4-phosphates of IP_3. The 1-phosphate is not deeply buried, explaining how, in the case of PtdIns-4,5-P_2 binding, linkage through the 1-phosphate to diacylglycerol is accomodated.

The crystal structure of the Bruton's tyrosine kinase (BTK) PH domain/IP_4 complex reveals similarities but crucial differences from the PLCδ structure (Baraldi et al., 1999). The IP_4 is located at an analogous position between the β1-β2 loop and the β3-β4 loop of the BTK PH domain, and the 1-phosphate is pointed out of the pocket to allow accomodation of the diacyglycerol moiety of PtdIns-3,4,5-P_3. However, the inositol ring is flipped over 180° such that the 3-phosphate occupies a position analogous to the 5-phosphate of IP_3 in the PLCδ structure. This places the 3-phosphate of IP_4 at the deepest part of the pocket in contact with Arg-28 (analogous to Arg-40 of PLCδ) and Lys-12 (analogous to Lys-30 of PLCδ). This structure explains why mutation of Arg-28 to Cys decreases the affinity of the BTK PH domain for PtdIns-3,4,5-P_3 but does not significantly affect the ability to bind to PtdIns-4,5-P_2 (Rameh et al., 1997b). The Arg-28 to Cys mutation was identified as a natural mutation causing a defect in B cell development and signaling, emphasizing the importance of the PtdIns-3,4,5-P_3 binding for BTK signaling in B cells. Mutation of the analogous Arg residue in other PtdIns-3,4,5-P_3-binding PH domains also compromises binding to PtdIns-3,4,5-P_3, suggesting that these domains also bind this lipid in the orientation found in BTK rather than the orientation found in PLCδ.

Alignments of the β1-β2 loop regions of Group I, II, III, and IV PH domains (Figure 2B) allows one to rationalize the lipid binding specificity with the primary structure. For example, the Group I, II, and III PH domains have conserved the two critical basic residues in the β1 and β2 strands (analogous to Lys-12 and Arg-28 of BTK). In contrast, Group IV PH domains, such as dynamin, do not have these residues conserved. This probably explains the relatively low affinity of these domains for phosphoinositides. The Group I PH domains have large loop regions between the β1 and β2 strands and have multiple basic residues in this loop region. The BTK crystal structure reveals that the ε amino group of Lys-18 in this loop forms a salt bridge with the 5-phosphate of IP_4 and that this phosphate is further stabilized by interactions with the backbone of the loop and by the local basic charge provided by Lys-17 and Lys-19 (Figure 2A).

Finally, the crystal structure of the BTK PH domain/IP_4 complex suggests that binding to PtdIns-3,4,5-P_3 on a membrane surface stabilizes BTK in a dimeric state (Figure 3A). Dimer formation could enhance transphosphorylation and activation of BTK, both of which depend on PI3K activation in vivo (Scharenberg et al., 1998). Interestingly, the crystal structure of the PH domain of an activating mutant of BTK (Glu-41 to Lys) revealed a second, low-affinity IP_4-binding site per monomer on the same face as the high-affinity site found on wild-type BTK (Figure 3B). Lys-41 of the mutant contributes a salt bond to the new IP_4 site.

In summary, the structural basis for recognition of specific phosphoinositides by FYVE domains and PH domains is beginning to emerge. It is now clear that multiple types of domains have independently evolved pockets for recognition of specific phosphoinositides as a means of regulated recruitment to membranes. In addition, a surprisingly large number of FYVE domain–containing proteins and PH domain–containing proteins with diverse functions are predicted to bind to PI3K products based on the structures discussed here. Whether all these proteins function in PI3K signaling pathways remains to be determined, yet it is clear that localization of signaling proteins at membrane sites where PI3K becomes activated is critical for many types of signaling networks.

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